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Aggregation Numbers and Shapes of Lysophosphatidylcholine and Lysophosphatidylethanolamine Micelles

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Aggregation numbers and shapes of 1-palmitoyl-sn-glycero-3-phosphocholine(LPPC) and 1-lauroyl-sn-glycero-3-phosphoethanolamine(LLPE) micelles have been investigated at 35 °C by static and dynamic light scattering method. The average aggregation number of LPPC micelle is 139. The LLPE micelle is rod-like shape, and grows with increasing concentration. At 5.87 mg cm^{-3} micelle concentration, apparent aggregation number of LLPE is 902 and average length of rigid rod is estimated to be 45-57 nm.

Lysophospholipids have different properties from the corresponding diacylphospholipids.[1-3] Their solubilities in water are higher than those of the respective diacyl compounds. They dissolve to form micelles. Both lysophosphatidylcholine(LPC) and lysophosphatidylethanolamine(LPE) have one hydrocarbon chain and a individual zwitterionic polar group. They play important roles in biological systems, and the roles are related to the geometric properties and the packing structures of molecules. In general, micelle shapes depend on the "critical packing parameter" of monomer.[4] Recently, micelle size of LPE was dynamically simulated.[5] However, micelle shapes and sizes of LPC and LPE have not yet been well characterized experimentally.

In this work, the static and dynamic light scattering have been measured for aqueous solutions of 1-palmitoyl-sn-glycero-3-phosphocholine(LPPC) and 1-lauroyl-sn-glycero-3-phosphoethanolamine(LLPE). Here, we use LLPE, because its Krafft temperature is about 27 °C and that of palmitoyl LPE is about 57 °C. The synthetic LPPC and LLPE samples were purchased from Sigma Chemical Co.,Ltd. and Avanti Polar Lipids, Inc., respectively. Their purities were checked by thin layer chromatography. The water used was obtained by distillation of an alkaline $KMnO_4$ solution. The static and dynamic light scattering were measured at 35 °C on an Otuka Electronics dynamic light scattering spectrometer DLS-700. The light source is an argon ion laser of a 488 nm wavelength(λ). The measurement of specific refractive index increment was also carried out on an Otuka Electronics differential refractometer RM-102 and performed at a 488 nm wavelength and 35 °C. Solvents and solutions were purified by filtering them through a Millipore membrane filter and filtration of LLPE solution was carefully carried out above 35 °C. The detailed procedures for the both static and dynamic light scattering have been described elsewhere.[6,7] On dynamic light scattering, a mutual diffusion coefficient D was evaluated by the cumulant method.

On static light scattering, the Debye plot at angle θ for dilute solutions of micelles is described by

$$K(c-c_0)/(R_\theta-R^0_\theta)=(1/M)(1+1/3R_G{}^2\mu^2)+2B_2(c-c_0) \qquad (1)$$
$$\mu^2=(4\pi\tilde{n}_0/\lambda)^2\cdot\sin^2(\theta/2)$$

for small $R_G{}^2\mu^2$. In Eq.(1) K is the optical constant, and R and R^0 are the reduced scattering intensities at surfactant concentration c and the critical micelle concentration c_0, respectively. M is the average molecular weight of micelles, R_G is the average radius of gyration of the micelle, μ is the magnitude of scattering vector, B_2 is the second virial coefficient and $\tilde{n}_0$ is the refractive index of water. When the reciprocal reduced scattering intensity $K(c-c_0)/(R_\theta-R^0_\theta)$ has no angular dependence or when the scattering angle is extrapolated to zero, Eq.(1) is written as follows:

$$K(c-c_0)/(R_0-R^0_0)=(1/M)+2B_2(c-c_0) \qquad (2)$$
$$\equiv 1/M_{app} \qquad (3)$$

where the subscript "app" means that the values are apparent.

The reciprocal scattering intensity exhibited no angular dependence for LPPC solution, indicating that the size of LPPC micelle is smaller than $\lambda/20$. Then all measurements for LPPC solutions were carried out at $\theta=90°$. On the other hand, the angular dependence for LLPE is not negligible. Figure 1 shows the angular dependence of static light scattering for the LLPE solution at 5.87 mg cm^{-3} micelle concentration. When dissymmetry is large, the reciprocal reduced scattering intensity at each concentration must be extrapolated to zero scattering angle.

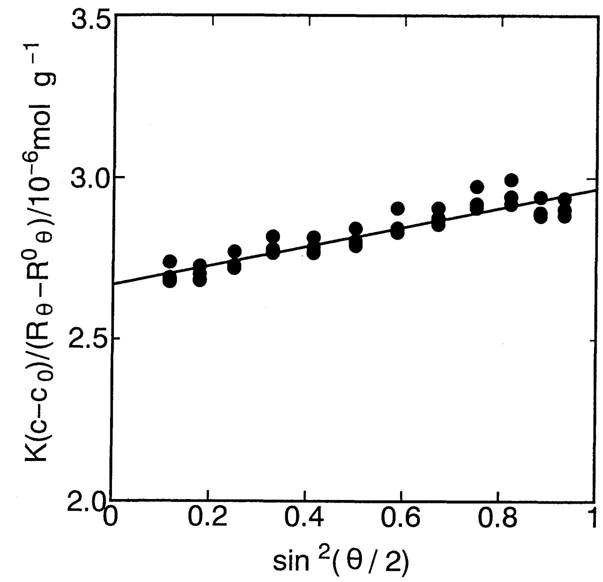

Fig.1. Angular dependence of reciprocal scattering intensity for solution of LLPE. Micelle concentration is 5.87 mg cm^{-3}.

Debye plots for LPPC at $\theta=90°$ and for LLPE at zero scattering angle are shown in Fig.2. The reciprocal scattering intensity for LPPC increases linearly with an increase in micelle concentration, $c-c_0$. This indicates that the micelle size of LPPC remains constant with increasing the micelle concentration. The micelle molecular weight of LPPC and the second virial coefficient are evaluated from Eq.(2) as 7.15×10^4 and 2.6×10^{-5} mol cm^3 g^{-2}, respectively. The micelle aggregation number of 139 was also obtained from the micelle molecular weight and monomer molecular weight. Furthermore, the small virial coefficient obtained here may suggest that weak repulsion is operative in the intermicellar interaction of LPPC.

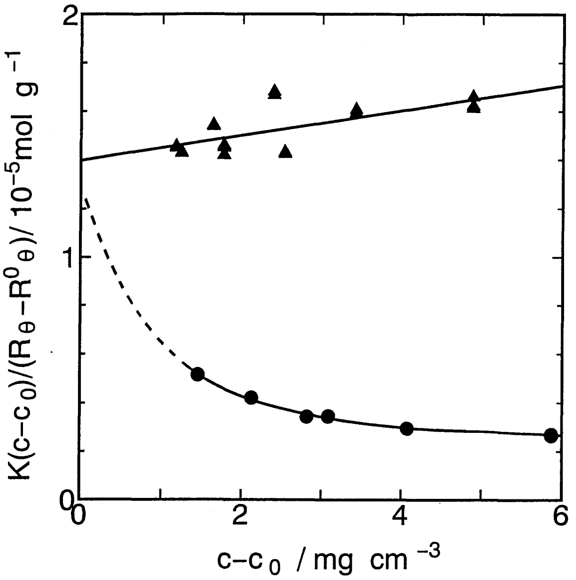

Fig.2. The Debye plots for solution of LPPC at θ=90°(▲) and of LLPE at zero scattering angle(●).

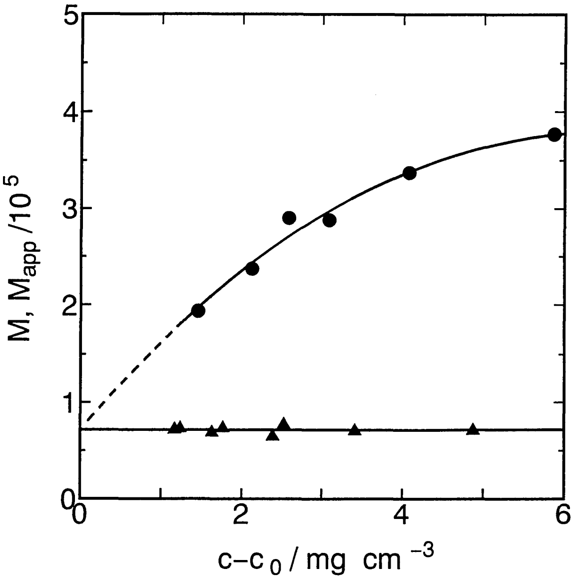

Fig.3. The average molecular weight(M) of LPPC micelle(▲) and the apparent average molecular weight(M_{app}) of LLPE micelle(●).

In Fig.2, a gradual decrease in the reciprocal scattering intensity for LLPE with an increase in micelle concentration is shown. It is suggested from Eq.(2) that with increasing concentration the effect of the growth of micelle size becomes greater than the effect of the intermicellar interaction. However, since it is not easy to obtain these two effects separately, the reciprocal value of $K(c-c_0)/(R_0-R^0_0)$ is evaluated as the apparent average molecular weight of the micelles given in Eq.(3) at each micelle concentration.

The variation of M_{app} of LLPE micelle and M of LPPC micelle are shown in Fig.3 as a function of the micelle concentration. It is seen that the LLPE micelle grows with increasing the concentration, and at a critical micelle concentration it will be close to the value of LPPC micelle. At 5.87 mg cm^{-3} micelle concentration, M_{app} of LLPE micelle is equal to 3.75×10^5, which gives the apparent average aggregation number 902, suggesting the formation of rod-like micelle.7)

On the dynamic light scattering, scattering intensity from the LPPC micelle solution is not sufficient to measure even in low scattering angles. This is consistent with the result obtained from the static light scattering that the micelle size of LPPC is small. For LLPE, the value of D obtained at 5.87 mg cm^{-3} micelle concentration and at θ=20° is 2.88×10^{-7} cm^2 s^{-1}. At the scattering angle higher than 30°, and/or at more dilute concentrations than 5.87 mg cm^{-3}, light scattering intensity is too low to be detected. For small $R_G{}^2\mu^2$, D is related to the total translational diffusion coefficient D_0, and the hydrodynamic radius of micelles R_H by Eqs.(5)

and (6)[7]

$$D=D_0[1+k_D(c-c_0)] \tag{5}$$
$$D_0=k_BT/6\pi\eta_0R_H \tag{6}$$

where k_D is the hydrodynamic virial coefficient, k_B is the Boltzmann coefficient, T is the absolute temperature and η_0 is the viscosity of solvent. Supposing the value of k_D to be -5 cm^3 m^{-1}, which is relatively small as a value of k_D for a rod-like micelle,[7] the value of R_H is evaluated to be 10.6 nm.

Assuming that the shape of LLPE micelle is rigid rod, the counter length L can be calculated from Eqs. (7) and (8)[8]

$$R_G=(L^2/12+r^2/2)^{1/2} \tag{7}$$
$$R_H=L/(2\sigma-0.19-8.24/\sigma+12/\sigma^2) \tag{8}$$
$$\sigma=\ln(L/r)$$

where r is radius of a rigid rod and is taken as 2.4 nm, referring to the half thickness of crystal bilayer for dilauroyl phosphatidylethanolamine.[9] Values of the radius of gyration R_G for LLPE micelle can be obtained by applying the slope given in Fig.1 at low scattering angles to Eq.(1). At 5.87 mg cm^{-3} the calculated R_G is 16.7nm, and then L is 58 nm. On the other hand L obtained from R_H is 45 nm. Both values are in fair agreement with each other. Therefore it seems reasonable to assume that the shape of LLPE micelle is rigid rod.

In conclusion, the average micelle size of LPPC is independent of the LPPC concentration, and the mean aggregation number is 139. In contrast, the apparent average micelle size of LLPE increases with increasing the LLPE concentration, and the rod-like micelles are formed. It is suggested that the monomer shapes of LPPC and LLPE reflect the difference in the micelle shapes.

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